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BIOLOGICAL CHARACTERISTICS OF ADENOCARCINOMA CELL LINE (HOUA-I) AND B-LYMPHOBLASTOID CELL LINE (HOUA-II) ESTABLISHED FROM THE SAME TISSUE OF ENDOMETRIAL ADENOCARCINOMA

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ABSTRACT

Human endometrial adenocarcinoma was cultured and endometrial adenocarcinoma cell line (HOUA-I) and B-lymphoblastoid cell line (HOUA-II) were established. HOUA-I line consists of cells of spindle-like, roundish, and polygonal shapes, arranged in epithelial pavement and proliferating in multilayers. Their doubling time is about 50 hours and plating efficiency is about 25%. The chromosomes show a wide aneuploid distribution, with a mode at 46, and no karyological abnormality. The cells can be transplanted on hamster and nude mouse to form poorly differentiated adenocarcinoma. The HOUA-I line was determined as the endometrial adenocarcinoma cell line. On the other hand, HOUA-II line consists of small roundish cells with cytoplasmic processes, and the cells proliferate as floating spherical aggregates of cells. Their doubling time is 60 hours, and the chromosomes of the stem cells are 46,XX,inv (20p+q−). They give positive results for Epstein-Barr virus nuclear antigen (EBNA) but negative result for EB-viral capsid antigen (EBVCA). Synthesis of immunoglobulin G (IgG) and c-myc gene amplification were recognized. The HOUA-II line was determined as B-lymphoblastoid cell line.

(Jikeikai Med J 36: 303 – 315, 1989)

Key words: endometrial carcinoma cell line, B-lymphoblastoid cell line, hormone, Immunoglobulin synthesis

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INTRODUCTION

Culture of endometrial adenocarcinoma is being undertaken extensively these days, and it is useful in judging the presence of sex-steroid hormone receptor, hormone responding ability, susceptibility against chemotherapeutic agent. We have established two cell lines of mutually completely different properties from an endometrial adenocarcinoma. They are an endometrial adenocarcinoma cell line (HOUA-I) and a B-type lymphoblastoid cell line (HOUA-II). We would like to report on the progress of establishment of the lines, identification of the cells, and cytobiological properties of the cells, especially hormone responding ability, presence of hormone receptor, producing ability of carcinoembryonic proteins, immunoglobulin (Ig) producing ability, and presence of c-myc gene-amplification accoring to the result of our study.

MATERIALS AND METHODS

1. Culture materials and techniques

A 55-year-old Japanese woman underwent radical hysterectomy and lymph node resection on Feb. 6, 1982. A portion of the endometrial tumor (Fig. 1; mixed type of well differentiated, moderately differentiated and poorly differentiated adenocarcinomas) was rinsed twice with culture medium, finely minced with scissors, digested with 600 Pronase Unit Dispase/ml (Gohdo-Shusei Co. Tokyo) for 30 minutes at 37°C, and centrifuged at 900g for 10 minutes. The sediment was resuspended in the culture medium, placed in 6-cm plastic dishes (Terumo, Co., Ltd, Tokyo), and incubated at 37°C in a humidified atmosphere containing 5% CO_2 in air. The culture medium used was Ham's F-12 (GIBCO, N.Y.) supplemented with 15% fetal calf serum (Flow Lab., Md.). Both epithelial cell sheets and floating cell clusters were observed in the initiation of the primary culture. The cells were dissociated by using 0.25% trypsin solution and cultured after a 1 : 2 dilution.

2. Morphology

The cultured cells were observed with phase-contrast microscopy. They were also fixed with 95% ethanol solution and stained with Papanicolaou, periodic acid-Schiff (PAS), mucicarmine, alcian blue, and peroxidase solution. The cells were also observed with the JEM 100B electron microscope. Details of the procedures were published previously[1].

3. Growth characteristics

In the case of HOUA-I, approximately 1×10^5 single suspended cells were placed into 3.5cm plastic dishes and cultured 10 days. A cell count was taken each day in three dishes using Erma counting chamber, and the growth curves are drawn as shown in Fig. 4. The culture medium was changed every day. The population doubling time

(DT) and the saturation density (SD) were determined by the growth curve. In the case of HOUA-II, approximately 7×10^5 single suspended cells were placed into 3.5 cm plastic dishes and cultured for 10 days. The growth curves were drawn as shown in Fig. 4.

4. Chromosome analysis

The cultured cells were treated with 0.1% trypsin solution for 15 seconds at room temperature, stained with 3% Giemsa solution and analyzed with G-band or Q-band karyotyping. Histograms of chromosome number were determined from more than 50 metaphase plates.

5. Heterotransplantation

Approximately 1×10^7 or 1×10^8 cells were transplanted into the subcutis of 3 BALB/c nude mice (6 week-old, Clea Japan Co., Tokyo). The tumors were examined histologically 6 weeks after transplantation, respectively.

6. Carcinoembryonic proteins in conditioned media

Carbohydrate antigen (CA125), tissue polypeptide antigen (TPA), alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), human chorionic gonadotropin (HCG), tumor antigen (TA-4), ferritin, β2-microglobulin, and neuron specific enolase (NSE) were examined. 5×10^5 cells were cultured in serum-free Ham's F-12 medium for 2 days, and the amounts of the carcinoembryonic proteins in the conditioned media were measured by RIA.

7. E2- and P-receptors assay

17β-estradiol (E2-) and progesterone (P-) receptors were assayed by the methods of Horwits and McGuire[3] with slight modification.

8. Hormone effects

E2 and P (Mochida Pharmaceutical Co., Ltd., Tokyo) were dissolved in ethanol and diluted to the concentration (1×10^{-11} to 1×10^{-4} M) with a serum-free HB101TM medium (Hana Medical Inc., Ca.). The final concentration of ethanol in the medium was 0.5%, which produced no effect on the cellular growth and morphological characteristics. 1×10^5 single-suspended cells were placed into 3.5 cm plastic dishes and each concentration of E2 or P was administered separately 24 hours after start of culturing. The cells were cultured for 3 days after the removal of hormones and cell count was done per three dishes. The ratio (number of cells in hormone-treated group/that in non-treated group) was calculated and dose/response curves were drawn as shown in Fig. 7. The WI-38 (normal human fibroblast strain) cells[3] were used as a control in this experiment.

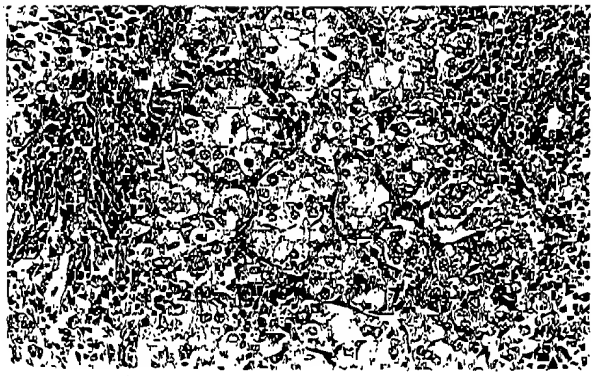
9. Identification whether or not HOUA-II is B-lymphoblastoid line

We examined the biological properties such as 1) phagocytosis, 2) immunoglobulin (Ig) synthesis, 3) surface marker (E, sheep erythrocytes; ox-EAC, ox erythrocytes coated with antibody and human complement and ox-EA-(IgG), ox erythrocytes coated with rabbit IgG specific for ox erythrocytes), 4) immunofluorescent staining with anti-B cell antibody (Leu 10) and anti-T cell antibody (Leu 5). 5) Epstein-Barr virus nuclear antigen (EBNA) and EB-viral capsid antigen (EBVCA), 6) terminal deoxynucleotidyl-transferase, and 7) c-myc gene amplification. The methods were previously reported[4].

RESULTS

1. Histology of culture materials

The tumor was interpreted pathologically as mixed type of adenocarcinoma (well differentiated, moderately differentiated and poorly differentiated types) (Fig. 1). The



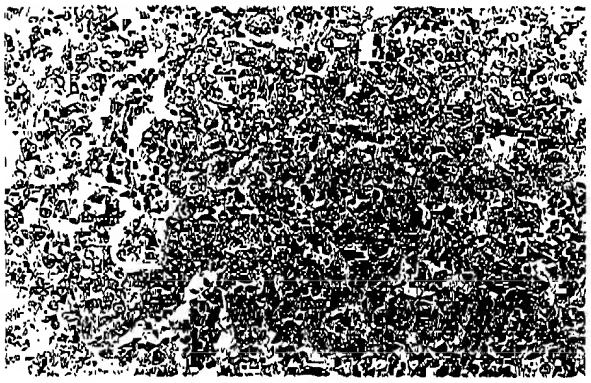


Fig. 1. Histology of the culture materials (endometrial tumor), showing adenocarcinoma (mixed with various degrees of differentiation; a, well differentiated type; b, moderately differentiated type; c, poorly differentiated type). Hematoxylin-eosin stain, ×100.

cytoplasms were moderately stained with PAS-digested diastase and negatively with mucicarmine and alcian blue.

2. Morphology of cultured cells

The HOUA-I cells were spindle, columnar and polygonal in shape and had an epithelial cell arrangement (pavement and jigsaw puzzle-like). They revealed a piling-up tendensy without contact inhibition and showed anaplastic and pleomorphic features (Fig. 2). The cytoplasms were stained with PAS and negatively with mucicarmine and alcian blue. Electron microscopy revealed that most individual cells were characterized by highly indented nuclei with multiple nucleoli. The cells had numerous microvilli, mitochondria, and free ribosomes. The adjacent cells were united with desmosomal junctions.

The HOUA-II cells were 10–20 μm in diameter and were uniformly round and oval in shape, and grew as single-cells in suspension or in groups of 20 to more than 100 cells in stationary culture (Fig. 3). Most cells had cytoplasmic processes. About 5% of cells were stained weakly with PAS, while all cells were not stained with mucicarmine, alcian blue, and peroxidase. The cells were characterized by smooth nuclear membrane and poorly developed organella except for rough-endoplasmic reticulum. Electron microscopy revealed no virus-like particle in these cells.

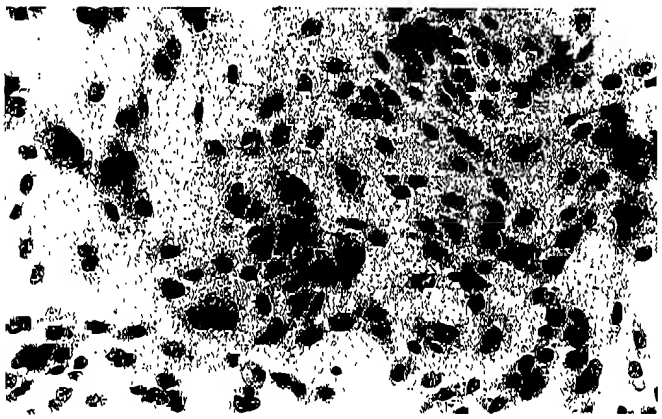


Fig. 2. Monolayer cultured cells of HOUA-I line. The cells show anaplastic, pleomorphic features and epithelial cell arrangement. Papanicolaou's stain, ×100.

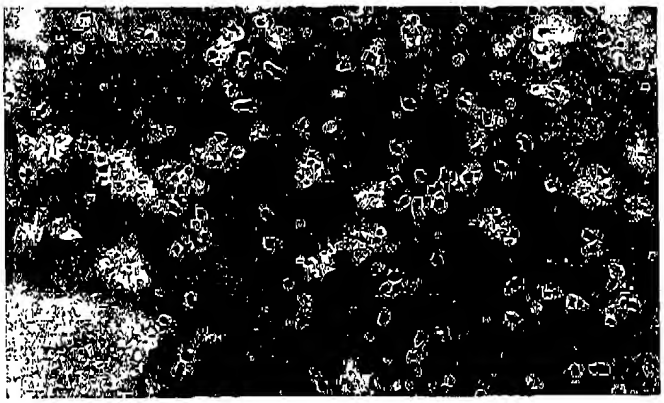


Fig. 3. The HOUA-II cells are uniformly round in shape and grow as cell clumps in the stationary culture. Some cells have cytoplasmic processes. Phase-contrast microscopy, ×100.

3. Growth characteristics

Both HOUA-I and HOUA-II line grew well and more than 150 serial passages were performed within 3 years. The growth characteristics such as GC (Fig. 4), DT, SD, and PE[1] are shown in Table 1. The DT was about 50 hours in the case of HOUA-I and 60 hours in the case of HOUA-II.

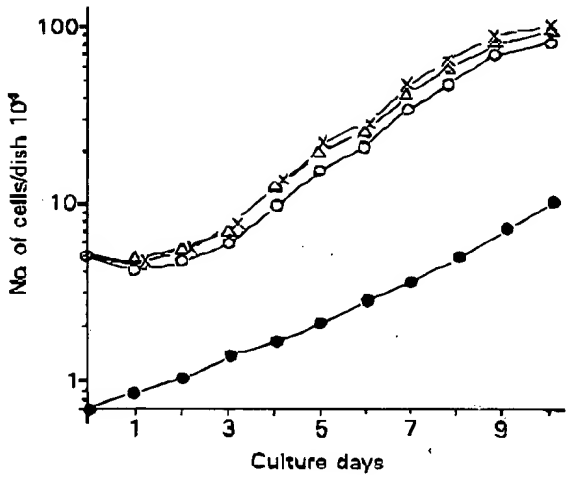


Fig. 4. Growth curves of passage 10 (○), passage 50 (△) and passage 100 (×) of HOUA-I, and that of passage 10 (●) of HOUA-II. The population doubling time of HOUA-I was about 50 hours and that of HOUA-II was 60 hours.

Table 1. Growth characteristics of HOUA-I and HOUA-II lines

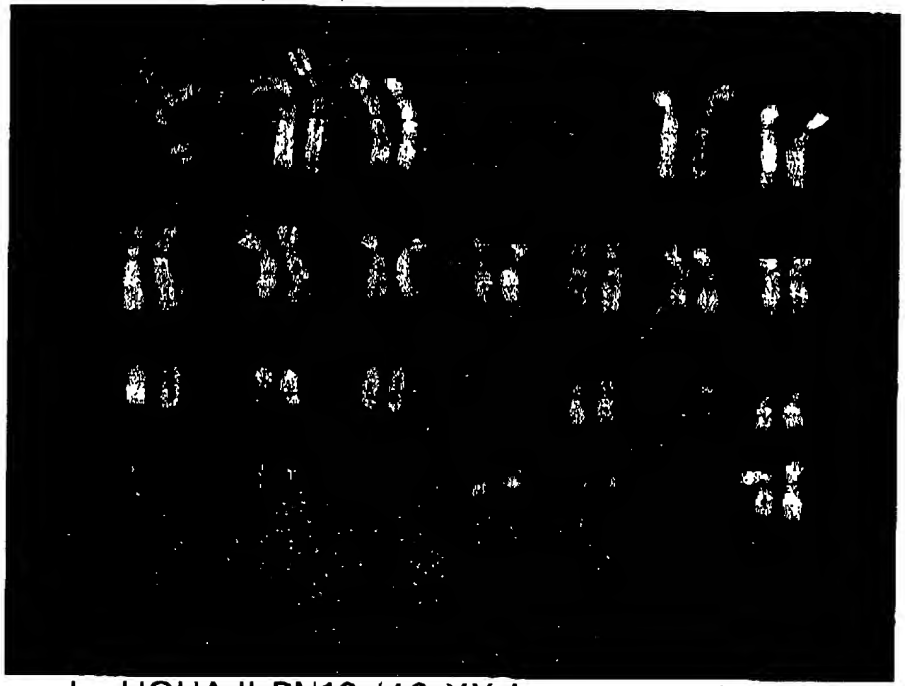
Cell line	PN	DT (hr)	SD ($\times 10^4/cm^2$)	PE (%)
HOUA-I	10	72	8.3	25
	50	50	8.5	27
	100	50	8.6	27
HOUA-II	10	62	nd	nd

Note. PN, passage number; DT, population doubling time; SD, saturation density; PE, plating efficiency[1]; nd, not done

4. Chromosome analysis

Chromosome number of HOUA-I line (passage 5, 50, and 100) was widely distributed and aneuploidy. However, the stem cells showed normal karyotype (Fig. 5a). On the contrary, chromosome number was concentrated in the diploid range, inv (20p+q−) being observed in all cells of HOUA-II line (Fig. 5b).

a. HOUA-I (46, XX)



b. HOUA-II PN10 (46, XX, inv (20p+q−))

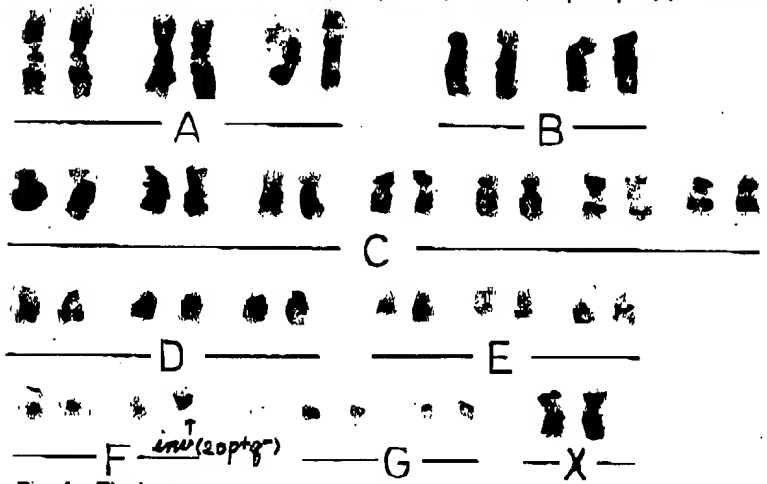


Fig. 5. The karyotype of HOUA-I (a, passage 20; Q-band) is normal, and that of HOUA-II (b, passage 20; G-band) shows karyological abnormality, inv (20p+q−).

5. Heterotransplantation

The HOUA-I cells were transplantable in nude mice, while HOUA-II cells were not. The tumors produced by HOUA-I were poorly differentiated adenocarcinoma (Fig. 6).

Fig. 6. Histology of tumor produced by heterotransplantation of HOUA-I cells into the subcutis of nude mouse showing poorly differentiated adenocarcinoma. Hematoxylin-eosin stain. ×100.

6. Carcinoembryonic proteins in the conditioned media

The HOUA-I cells produced such carcinoembryonic proteins as CA125 (146 U/ml), TPA (1500 U/ml), CEA (5.8 ng/ml), and ferritin (5.1 ng/ml), while AFP, HCG, NSE, TA-4, and β2-microglobulin were not detected in the culture media. The HOUA-II cells did not produce any carcinoembryonic proteins.

7. E2- and P- receptors

These receptors were not detected in both HOUA-I and HOUA-II.

8. Effects of hormone on proliferation

E2 enhanced slight proliferation at 1×10^{-7} M, whereas it suppressed proliferation at high concentrations of more than 1×10^{-5} M in the case of HOUA-I (Fig. 7a). On the contrary, P suppressed proliferation at high concentrations of more than 5×10^{-6} M in the case of HOUA-I (Fig. 7b). E2 and P suppressed the rate of growth of HOUA-II cells and the WI-38 cells at 5×10^{-5} M (Figs. 7a and 7b).

9. Phagocytosis

Phagocytosis of ink granules was not observed in all these cell lines.

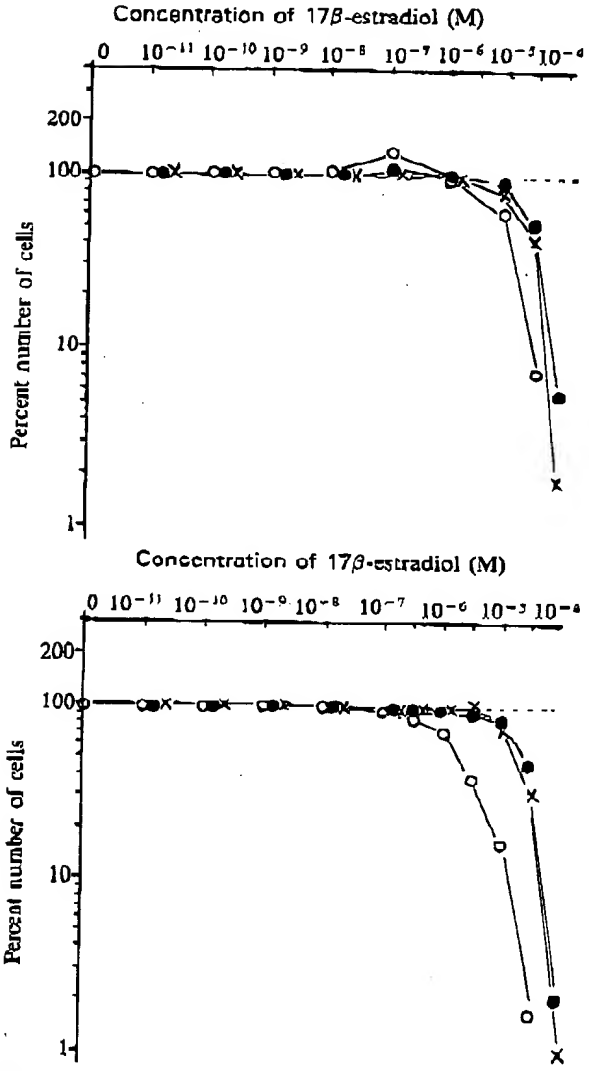


Fig. 7. (a) Effects of 17β-estradiol on cell proliferation of HOUA-I cells (○), HOUA-II cells (●) and WI-38 cells (×). The growth of HOUA-I cells is slightly promoted by 10^{-7} M 17β-estradiol. (b) Effects of progesteron on cell proliferation of HOUA-I cells (○), HOUA-II cells (●) and WI-38 cells (×). Growth of HOUA-I cells is strongly suppressed with an increase of progesterone. Both 17β estradiol and progesterone did not affect on the proliferation of HOUA-II and WI-38 cells at 10^{-11} –10^{-6} M.

10. Synthesis of Ig

The HOUA-II cells synthesized IgG (γ, χ), but the HOUA-I cells did not synthesize Igs (Table 2).

Table 2. Immunoglobulin Synthesis, Surface Receptor, and EB Virus

Cell	Immunoglobulin					Surface receptor			EB virus		
line	γ	μ	α	χ	λ	E	ox-EAC	ox-EA (IgG)	EBNA	EBVCA	VP
HOUA-I	−	−	−	−	−	−	−	−	−	−	−
HOUA-II	+	−	−	+	−	−	+	+	+	−	−

Note. E=sheep erythrocyte, ox-EAC=ox erythrocytes coated with antibody and human complement, ox-EA (IgG)=ox erythrocytes coated with rabbit IgG specific for ox erythrocyte, EBNA=Epstein-Barr virus associated nuclear antigen, EBVCA=EB-viral capsid antigen, VP=virus particle

11. Surface markers

E, ox-EAC, and ox-EA-(IgG) receptors were examined. These receptors were not detected in HOUA-I line. In the case of HOUA-II line, E receptor was not detected, while ox-EAC receptors were detected in about 40% of cells, and ox-EA-(IgG) receptors were detected in about 60% of cells (Table 2). The fluorescent anti-B cell antibody (Leu 10) was detected in about 60–70% of HOUA-II cells, while the fluorescent anti-T cell antibody (Leu 5) was not detected. These antibodies were not detected in HOUA-I cells.

12. EB-NA and EB-VCA

EB-NA was detected (Fig. 8), while EBVCA was not in all cells of HOUA-II line. These parameters were not detected in HOUA-I cells.

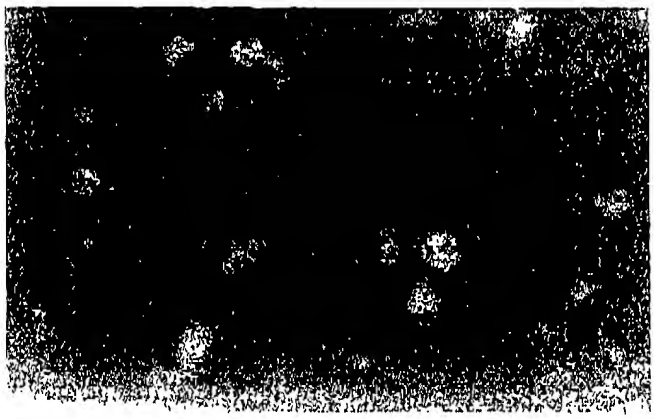


Fig. 8. Ebstein-Barr virus associated nuclear antigen is detected in the nuclei of HOUA-I cells. Immunofluorecent microscopy. ×200.

13. c-myc amplification

The c-myc amplified ca 10-fold in the HOUA-II line and encorded 1.3 kilobase Eco RI genomic DNA fragments.

DISCUSSION

We have established two cell lines of mutually, completely different nature from a human endometrial adenocarcinoma. They are a system showing adhering proliferation (HOUA-I) and one showing floating proliferation (HOUA-II). We have investigated the cytobiological properties of the lines and identified them.

HOUA-I line was identified to be an endometrial adenocarcinoma cell line based on their properties as follows: 1. The cells are epithelial cells, and the nuclei are highly neoplastic and pleomorphic. 2. No contact inhibition takes place, and the cells proliferate in multi-layers. 3. The chromosomes show a wide aneuploid distribution. 4. The cells produce carcinoembryonic proteins. 5. Heterotransplantation can form a poorly differentiated adenocarcinoma. 6. The cells have a responding ability against sex-steroid hormones. The effects of E2 and P on cell growth in this study were assesed with the use of target cells (endometrial carcinoma cells) as well as nontarget cells (WI-38). The growth-stimulating effects of E2 at the 1×10^{-7} M and the growth suppressing effect of P at the 5×10^{-6} M were considered as the hormone specific effects, because the WI-38 cells were not effected by these hormones at the concentration of 5×10^{-5} M.

On the other hand, identification of HOUA-II line was difficult, but it was fianally identified as a B-lymphoblastoid line as shown by the following data: 1. The cells proliferated in flotation. 2. Receptor-negative reaction against sheep red blood cell. 3. Positive complement receptor reaction. 4. Positive IgGFc receptor reaction. 5. Positive EBNA reaction. 6. Positive human B cell antibody (Leu 10). 7. Negative human T cell antibody (Leu 5). 8. No phagocytic ability. 9. Negative peroxidase staining reaction. 10. Presence of synthesizing IgG. 11. Abnormality in karyotype. 12. Passages for as long as 5 years or more. 13. Negative in terminal deoxynucleotidyl-transferase activity. Minowada *et al.*[5] classified lymphoblastoid lines into T cell type and B cell type. The characteristics of the HOUA-II line are in accord with those of B-lymphoblastoid line mentioned by Minowada.

It has been well-known that EB virus is capable to transform lymphocytes into lymphoma cells and that the target cell is B cell[6]. The fact that HOUA-II line is positive against EB-NA, while HOUA-I line and the culture material were negative, is explained to be due to EB virus infecting the B-lymphocytes contained in the carcinoma tissue to change into blastoma in *in vitro* system.

There is no report, as we are aware, that the cancer cell line and B-lymphoblastoid cell line were established from the same culture material at the same time. These cell lines are expected to be used as a material for studying 1) the interaction in *in vitro* between cancer cells and B-lymphoid cells, and 2) for manufacturing a monoclonal antibody.

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